Supplementary Figure 4

a

b

Lateral motion during experiment

c

Corrected vs. uncorrected Ca²⁺-traces

d

Correlation corrected vs. uncorrected Ca²⁺-traces
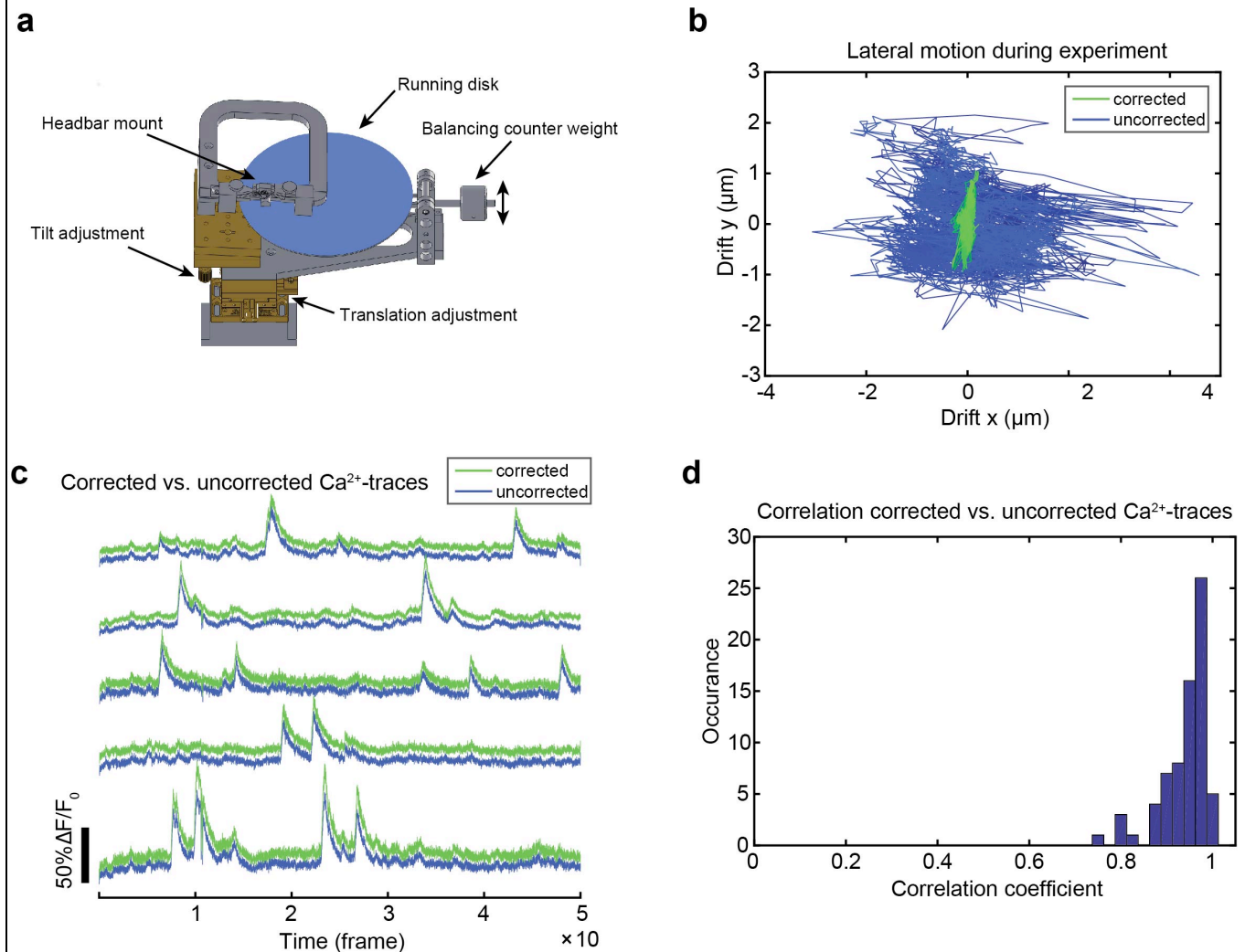

Supplementary Figure 4

Custom animal mount minimizes brain motion during awake imaging.

(a) Schematics of the custom animal mount utilized in the experiments. The mouse is head restrained but can freely move on a rotating disk. The disk is suspended by springs and a damped counter weight and the animal is held up by a custom jacket (see Fig. 2b). These measures reduce and compensate for any force that is applied by the mouse's limbs and thus minimize the vertical motion of the mouse brain during active behavior **(b)** Typical measured lateral drift of the images during the experiment before (blue) and after (green) motion correction in image postprocessing. In general, motion is <5µm r.m.s before and <1µm after correction. Dataset is the same as in Fig. 2c, motion correction algorithm is based on tracking the peak of the image autocorrelation using a maximum likelihood estimation algorithm and subsequent sub-pixel shifting using image interpolation. The high frame rate of s-TeFo (160 Hz) further facilitated image motion correction, as the in-frame movement during the acquisition of a single frame becomes negligible. **(c,d)** Representative calcium traces extracted before (blue) and after (green) motion correction in image postprocessing. Note that the curves are deliberately offset vertically from each other. **(e)** Histogram of correlations between corrected and uncorrected traces. On average the correlations are high (R=0.94±0.05). Frame rate is ~160Hz.